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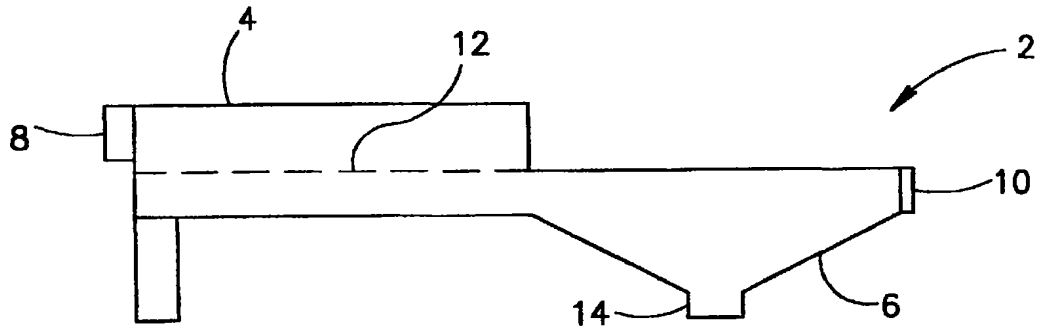

(57) **Abstract:** A method for preventing the transfusion of incompatible donor blood from a blood unite to a recipient. The method comprises the following steps: (a) connecting the recipient to the blood unit through an infusion tubing including an on-line device; (b) transferring a blood sample from the recipient into the separating chamber of the device; (c) obtaining the liquid component from the recipient's blood sample in the separating chamber; (d) combining the liquid component with the donor blood in the testing chamber of the device; and (e) observing the reaction of the recipient of the recipient's liquid component with the donor blood, the presence of an agglutination reaction indicating that the donor blood is incompatible with the recipient. The device comprises (i) a separating chamber for separating a liquid component from the recipient's blood; and (ii) a testing chamber in fluid communication with the separating chamber for combining the recipient's liquid component and the donor's blood, the contents of the testing chamber being observable from outside the chamber.

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BLOOD TRANSFUSION METHOD AND DEVICE

FIELD OF THE INVENTION

This invention relates to a method and device for determining blood transfusion compatibility.

BACKGROUND OF THE INVENTION

The following references are considered as possibly being relevant to understanding the invention, but have no influence on the patentability of the invention:

1. Lumadue, J.A., Boyd, J.S. and Ness, P.M., Adherence to a strict specimen-labeling policy decreases the incidence of erroneous blood grouping of blood bank specimens, *Transfusion*, **37**:1169-1172.
2. U.S. Patent No. 4,685,314.
3. Jensen, N.J. and Crosson, J.T., An automated system for bedside verification of the match between patient identification and blood unit identification, *Transfusion*, **36**:216-221, 1996.
4. EP 634,216
5. EP 741,296
6. Fricker, J., Conversion of red blood cells to group O, *The Lancet*, 347;680, 1996.

Blood transfusion is a well-accepted technique used the world over. Blood banks that store donated blood units provide the blood units on request for transfusion into patients requiring the blood. However, life-threatening hemolytic transfusion reactions can and do occur when there is a mismatch between the donor

and recipient blood types. It is believed that ABO-incompatible transfusions due to the misidentification of donor or recipient samples kill as many as 24 patients in the United States each year (AuBuchon, J.P. and Kruskall, M.S. (1997) *Transfusion* 37:1211). It is believed that many more cases are unreported, for obvious reasons. The great majority of blood type mismatches are believed to occur as a result of human error.

In order to prevent blood type mismatches, blood crossmatch tests are routinely carried out with the recipient's plasma or serum and the donor's blood (whole blood or packed cells). The conventional way of separating plasma or serum from whole blood is by centrifugation.

A number of approaches have been suggested in order to prevent blood type mismatches:

1. Enforcing strict requirements regarding testing, labeling and identification of donated blood units and patients' blood (1).
2. Mechanical devices which are attached to the donated blood bag after the crossmatch test has been carried out, and which physically allow transfusion only after complete identification of the recipient. An example of this approach is disclosed in (2), which describes the use of a locking mechanism on the blood bag corresponding to the blood type of the bag. The patient is provided with a key corresponding to his blood type, so that the patient can receive a transfusion only from a blood bag which can be opened by his key.
3. Bedside verification of the compatibility between patient and blood unit. This could take the form of a portable bedside barcode scanner and computer (3), or by retesting the compatibility by separating the patient's plasma and carrying out an agglutination reaction using various reagents and a centrifuge (e.g. (4)). (5) discloses a pre-transfusion card which has a series of zones, some impregnated with dried reagents and others for receiving different mixtures of donor and recipient blood.
4. The enzymatic conversion of all red blood cells to type O (6).

All of the above approaches have various disadvantages which will be obvious to the skilled artisan. A need therefore exists for a simple and inexpensive method for determining blood transfusion compatibility.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a method for preventing the transfusion of incompatible donor blood, which can be carried out at bedside without the use of a centrifuge.

It is a further object of the invention to provide a device for detecting an incompatibility reaction between donor blood and a recipient.

In a first aspect of the invention, there is provided a method for preventing the transfusion of incompatible donor blood from a blood unit to a recipient comprising:

(a) connecting the recipient to the blood unit through an infusion tubing including an on-line device, the device comprising:

i) a separating chamber for separating a liquid component from the recipient's blood; and

ii) a testing chamber in fluid communication with the separating chamber for combining the recipient's liquid component and the donor's blood, the contents of the testing chamber being observable from outside the chamber;

(b) transferring a blood sample from the recipient into the separating chamber;

(c) obtaining a liquid component from the recipient's blood sample in the separating chamber;

(d) combining the liquid component with the donor blood in the testing chamber; and

(e) observing the reaction of the recipient's liquid component with the donor blood, the presence of an agglutination reaction indicating that the donor blood is incompatible with the recipient.

In a second aspect of the invention, there is provided a device for detecting an agglutination reaction between a liquid component of a first blood sample and red blood cells of a second blood sample comprising:

(a) a separating chamber for separating the liquid component from the first blood sample; and

(b) a testing chamber in fluid communication with the separating chamber for combining the liquid component and the second blood sample, the contents of the testing chamber being observable from outside the chamber;

wherein the device is capable of being connected on-line to an infusion tubing.

The method of the invention may be carried out at bedside without the use of expensive or complicated laboratory equipment. Furthermore, personnel having minimal training may carry out the invention. This enables the method to be carried out outside a hospital setting. The fact that the device is on line with the infusion tubing ensures minimal contact with the blood by health personnel.

In a third aspect of the invention there is provided a method for preventing the transfusion of incompatible donor blood to a recipient comprising:

(a) obtaining a blood sample from the recipient, the sample containing presumptive anti-blood group antibodies;

(b) contacting the sample with means which bind the antibodies, thereby binding the antibodies to the means;

(c) subsequently contacting the means with the donor blood; and

(d) observing the reaction of the donor blood with the means, the binding of the donor blood to the means indicating that the blood is incompatible with the recipient.

The means which bind the antibodies may be any known means, non-limiting examples of which are given below.

In a fourth aspect of the invention, there is provided a device for detecting an immunological reaction between anti-blood group antibodies of a first blood

sample and red blood cells of a second blood sample comprising means which bind the antibodies.

In the third and fourth aspects of the invention, the "*blood sample*" includes whole blood, plasma and serum.

BRIEF DESCRIPTION OF THE DRAWINGS

In order to understand the invention and to see how it may be carried out in practice, a preferred embodiment will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Fig. 1 is a schematic sectional front view of one embodiment of a device according to the invention;

Fig. 2 is a sectional side view of an optical device which may be used with the device of Fig. 1 in one embodiment of the method of the invention;

Fig. 3 is a sectional side view of another embodiment of a device according to the invention;

Figs. 4A, 4B and **4C** illustrate different stages in one embodiment of the method of the invention using blood group antigens bound to particles;

Figs. 5A, 5B and **5C** illustrate different stages in another embodiment of the method of the invention using anti-IgG antibodies bound to particles;

Fig. 6 is a sectional side view of an optical device which may be used with the device of Fig. 3 in a further embodiment of the method of the invention;

Figs. 7A, 7B and **7C** illustrate different stages in a still further embodiment of the method of the invention using blood group antigens bound to a planer surface;

Figs. 8A, 8B and **8C** illustrate different stages in another embodiment of the method of the invention using anti-IgG antibodies bound to a planer surface;

Figs. 9A and **9B** illustrate an additional device according to the invention indicating compatibility (Fig. 9A) and incompatibility (Fig. 9B); and

Figs. 10A, 10B and **10C** illustrate different stages in another embodiment of the method of the invention using the device of Fig. 9 and anti-IgG antibodies bound to magnetic beads.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Example I

One embodiment of the method of the invention will be described with reference to Fig. 1, which illustrates an on-line incompatibility detection device, generally indicated as 2. The device 2 comprises a separating chamber 4 in fluid communication with a testing chamber 6. The separating chamber has an inlet port 8 for receiving a first blood sample from a blood transfusion recipient, and the testing chamber has an inlet port 10 for receiving a second blood sample from the donor blood unit. Both inlet ports 8 and 10 may be connected by infusion tubing to the recipient and blood unit, respectively.

Although the device illustrated in Fig. 1 shows the separating chamber and testing chamber as integral parts of the same device 2, it will be understood that the two chambers may be separate, as long as they are in fluid communication.

The function of the separating chamber is to obtain a liquid component, generally in the form of plasma or serum, from the whole blood of the recipient. Some non-limiting possibilities for separating plasma or serum from the recipient's whole blood include:

a. Filtration through a commercial blood separating filter (for example Hemasep V of Pall Gelman, CPS-10 of Travenol or Plasmaflux P2 of Flesenius).

b. Blood coagulation or immunological binding of red blood cells to a matrix, followed by filtration through a 10-40 micron mesh.

In the illustrated embodiment of Fig. 1, the chamber 4 contains a filter 12 for separating plasma from the whole blood of the recipient. The chamber may be sealed under weak vacuum conditions, so designed to pull a fixed amount of blood

into the chamber. Commercial filters for plasma separation yield approximately 20% of blood volume as liquid plasma.

The function of the testing chamber is to combine the recipient's liquid component and the donor's blood in an agglutination test. In a preferred embodiment, the testing chamber has a conically shaped, flat bottom well 14. The testing chamber is connected to both the separation chamber and the donor blood bag. The volumes of recipient's plasma and donor's blood that are allowed to enter the testing chamber through separate ports are prefixed, and may be in the range of 0.02-0.2 ml.

One embodiment of the method of the invention begins with connecting the separation inlet port 8 of the device 2 to an injection apparatus (e.g. Venflon) and the testing inlet port 10 to the donor's blood bag. Each port contains a valve to control fluid flow therethrough. Opening the valve in the separation inlet port allows a fixed volume of the recipient's blood into the separation chamber 4. Plasma is separated from the blood through the filter 12 and flows into the testing chamber 6. An alternative for the separation inlet port valve may be puncturing the separation chamber with a commercial device such as a Vacuette Holder manufactured by Greiner.

Opening the valve of the testing inlet port 10 allows a fixed volume of the donor's blood into the testing chamber. After a gentle shaking of the chamber for good mixing of the recipient's plasma with the donor's blood, the device is left unmoved for several minutes, preferably at room temperature. The result of the test may be detected by visual (presence of a red spot on the conical bottom of the testing chamber) or optical (continuous transmittance reading device) means. The visual determination of agglutination may take approximately 10 minutes, while the optical determination may take 30-120 seconds.

Fig. 2 shows one embodiment of the invention, in which the result of the agglutination reaction is read by an optical reading device 16 which has a cavity 17 into which the detection device may be inserted. Whereas the detection device may be disposable, the optical reading device is generally for long-term multiple-use.

The optical device 16 consists of a light source 18, and an electro-optical sensor 20 positioned opposite the light source. An opaque door 22 at the entrance of the cavity prevents stray light from outside the device reaching the sensor.

The sensor monitors the agglutination reaction by reading the optical density of the suspension in the agglutination reaction chamber in two or more different locations (such as the conical bottom well of the chamber or proximate thereto), at several time intervals at the same location or by a combination of multiple readings at different time intervals and locations. The measurement may be made at the completion of the agglutination reaction or during the reaction. The signal from the sensor is transmitted to an electronic processor that displays the result by activating an audio or visual indicator 24, e.g. lights to indicate that an agglutination reaction has occurred (red) or that no reaction has taken place (green).

Optional components of the device include a physical connection between the donor's blood bag and the recipient's injection site with an automatic electronic valve that is controlled by the same electronic processor that activates the indicators. Thus, automatic initiation of infusion upon a negative result (i.e. no agglutination) is possible by using the optical reader device.

Example II

An embodiment of the third aspect of the invention is illustrated in Fig. 3, which shows a detection device 30 comprising an upper syringe 32 having an outlet port 34 in communication with a lower disposable separation column 36. The separation column contains means, in the form of a matrix 38, which binds anti-blood group antibodies. Antibody binding matrices are well known in the art. One example is illustrated in Figs. 4A-4C. Polymeric beads 42 are covered with a low-density layer of A and B blood group antigens 44 at a concentration (or distribution) which allows the antibodies 46 to bind with only one Fab arm 48 to the beads.

In a second example illustrated in Figs. 5A-5C, the beads 42 are covered with a high-density layer of anti IgG antibodies 52 (Fig. 5A). The anti-IgG

antibodies bind the F_c portion 54 of the recipient's antibodies 56 (Fig 5B). Alternatively, proteins which bind F_c fragments of human antibodies may be used.

In this embodiment of the invention, a sample of the recipient's blood (or plasma/serum) is injected into the column 36 from the syringe 32 (Fig. 3) and washed with saline. If the recipient's plasma contains any A and/or B antibodies, they will bind to the antigens or anti IgG antibodies of the matrix 38 and become attached to the beads. All other components of the recipient's blood or plasma are washed away. A small amount of the donated blood is then injected into the column and washed with saline. If the donor's red blood cells 58 have antigens that match the recipient's antibodies, these red blood cells will be bound by those antibodies and become attached to the beads (Figs. 4C and 5C), resulting in the appearance of a red color in the column. In this embodiment, the appearance of a reddish color in the column indicates an ABO incompatibility, and gives a NO-GO sign for the transfusion.

In another embodiment, an optical reading device 60, similar to the device illustrated in Fig. 2 above, can be used to detect the presence of red blood cells in the column (Fig. 6). The device may make single or multiple readings, providing that the beads are transparent (e.g. glass beads). A combination of beads and particles may also be used.

Example III

A further embodiment of the second aspect of the invention is illustrated in Figs. 7A-7C and 8A-8C. Instead of a column, the means which bind the antibodies may be in the form of blood group antigens or the anti-IgG antibodies which are bound to a solid matrix, e.g. a dipstick. Alternatively, substances such as proteins which bind F_c fragments of human antibodies may be used. The method is essentially the same: a solid matrix 70 is covered with highly diluted A 72 and B 74 antigens (Fig. 7A) or with concentrated anti-IgG antibodies 76 (Fig. 8A), as is well known in the art. The stick is dipped in the patient's blood or plasma/serum and subsequently washed in saline. A and B antibodies 78 of the patient's blood, if

present, bind to the matrix (Figs. 7B and 8B). A drop of the donor's blood is then contacted with the matrix and subsequently washed away with saline. If the donor's blood contains antibodies to the recipient's blood, his/her red blood cells 80 will bind to the recipient's antibodies which are already attached to the matrix (Figs. 7C and 8C) and the matrix or stick will become reddish (NO-GO sign). A visual determination in this process may be substantially immediate. Determination of the test results by an optical reader and, optionally, activation of an electronic valve controlling the blood infusion (as described above) is also contemplated as an embodiment of the invention.

Example IV

Figs. 9A-9D illustrate a still further embodiment of the invention which combines elements of the previous approaches. The device of this embodiment comprises a plasma separation chamber (not shown) and a conical testing chamber 82 as described above, but the testing chamber contains magnetic beads 84 bound by either A and B blood group antigens or by anti IgG antibodies 86 (Fig. 10A). The term "*magnetic*" encompasses any material with magnetic properties, i.e. attracted by a magnet or acting as a magnet. Alternatively, substances such as proteins which bind F_c fragments of human antibodies may be used. The recipient's plasma is added to the testing chamber 82 so that any antibodies 88 present in the plasma may be bound by the metal beads (Fig. 10B). A sample of the donor's blood is then added, with the testing chamber being gently shaken after each sample addition.

The testing chamber is then put on a magnetic base 90 whose upper surface corresponds to the bottom wall of the testing chamber, and the magnetic beads are concentrated to the bottom center 91 of the conical chamber. The appearance of a red spot 92 in the center (Figs. 9C and 9D) indicates ABO incompatibility (the donor's red blood cells 94 are attached to the beads 84 (Fig. 10C)) and gives a NO-GO sign for the transfusion. Compatible blood will not bind to the magnetic

beads, resulting in an evenly distributed red color 96 in the chamber (Figs. 9A and 9B). Here also, the reading may be executed visually or by an optical reader.

Non-magnetic beads which precipitate may also be used.

Although several embodiments have been described above, it will be obvious to the skilled artisan that various modifications and variations may be contemplated within the scope of the invention, which is defined by the following claims.

CLAIMS:

1. A method for preventing the transfusion of incompatible donor blood from a blood unit to a recipient comprising:

(a) connecting the recipient to the blood unit through an infusion tubing including an on-line device, said device comprising:

i) a separating chamber for separating a liquid component from the recipient's blood; and

ii) a testing chamber in fluid communication with said separating chamber for combining the recipient's liquid component and the donor's blood, the contents of said testing chamber being observable from outside the chamber;

(b) transferring a blood sample from said recipient into said separating chamber;

(c) obtaining the liquid component from the recipient's blood sample in the separating chamber;

(d) combining said liquid component with said donor blood in the testing chamber; and

(e) observing the reaction of the recipient's liquid component with the donor blood, the presence of an agglutination reaction indicating that the donor blood is incompatible with the recipient.

2. A method according to Claim 1 wherein said liquid component is plasma or serum.

3. A method according to Claim 1 wherein said separating chamber comprises a filter capable of separating plasma from blood.

4. A method according to Claim 1 wherein said separating chamber comprises a means for binding red blood cells to a matrix and a filter for separating between the bound red blood cells and the remaining plasma.

5. A method according to Claim 1 wherein said agglutination reaction is observed by an unaided eye.

6. A method according to Claim 1 wherein said agglutination reaction is observed by an optical reader.

7. A device for detecting an agglutination reaction between a liquid component of a first blood sample and red blood cells of a second blood sample comprising:

(a) a separating chamber for separating said liquid component from the first blood sample; and

(b) a testing chamber in fluid communication with said separating chamber for combining the liquid component and the second blood sample, the contents of said testing chamber being observable from outside the chamber;

wherein said device is capable of being connected on-line to an infusion tubing.

8. A device according to Claim 7 wherein said separating chamber comprises an inlet port for receiving the first blood sample and said testing chamber comprises an inlet port for receiving said second blood sample.

9. A system for detecting an agglutination reaction between a liquid component of a first blood sample and a second blood sample comprising a device according to Claim 7 and an optical reader capable of holding said device and detecting and displaying the optical density of the contents of said testing chamber.

10. A method for preventing the transfusion of incompatible donor blood to a recipient comprising:

(a) obtaining a blood sample from said recipient, said sample containing presumptive anti-blood group antibodies;

(b) contacting said sample with means which bind said antibodies, thereby binding said antibodies to said means;

(c) subsequently contacting said means with said donor blood; and

(d) observing the reaction of the donor blood with the means, the binding of the donor blood to said means indicating that the blood is incompatible with the recipient.

11. A method according to Claim 10 wherein said means which bind said antibodies are selected from the group consisting of blood group antigens, anti-IgG antibodies and proteins which bind F_c fragments of human antibodies.

12. A method according to Claim 11 wherein said means are bound to a solid matrix.

13. A method according to Claim 12 wherein said solid matrix is a plurality of beads, a plurality of particles, a dipstick, or their combination.

14. A method according to Claim 13 wherein said beads are magnetic.

15. A method according to Claim 13 wherein said particles are held in a column.

16. A method according to Claim 13 wherein said beads are placed in a testing chamber for combining the recipient's blood sample and the donor's blood, the contents of said testing chamber being observable from outside the chamber.

17. A method according to Claim 10 wherein said agglutination reaction is observed by an unaided eye.

18. A method according to Claim 10 wherein said agglutination reaction is observed by an optical reader.

19. A device for detecting an immunological reaction between anti-blood group antibodies of a first blood sample and red blood cells of a second blood sample comprising means which bind said antibodies.

20. A device according to Claim 19 wherein said means which bind said antibodies are blood group antigens and/or anti-IgG antibodies.

21. A device according to Claim 20 wherein said means are bound to a solid matrix.

22. A device according to Claim 21 wherein said solid matrix is a plurality of beads or a stick, or their combination.

23. A device according to Claim 22 wherein said beads are magnetic.

24. A device according to Claim 23 comprising a testing chamber containing said beads, and a corresponding magnetic base on which said chamber is mounted.

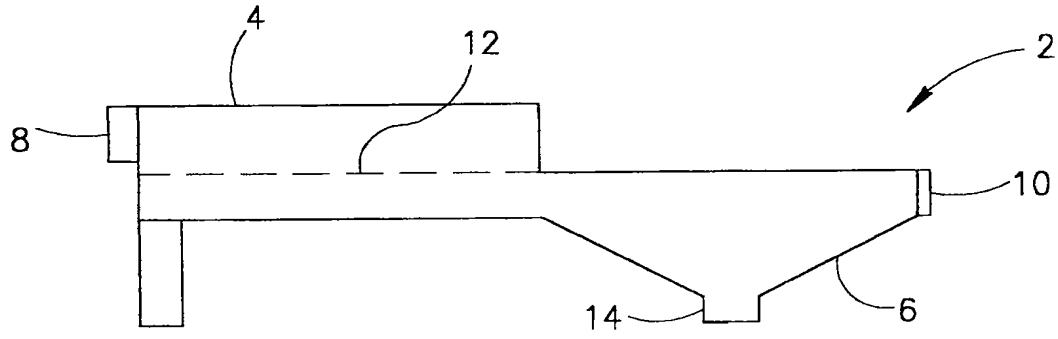

FIG.1

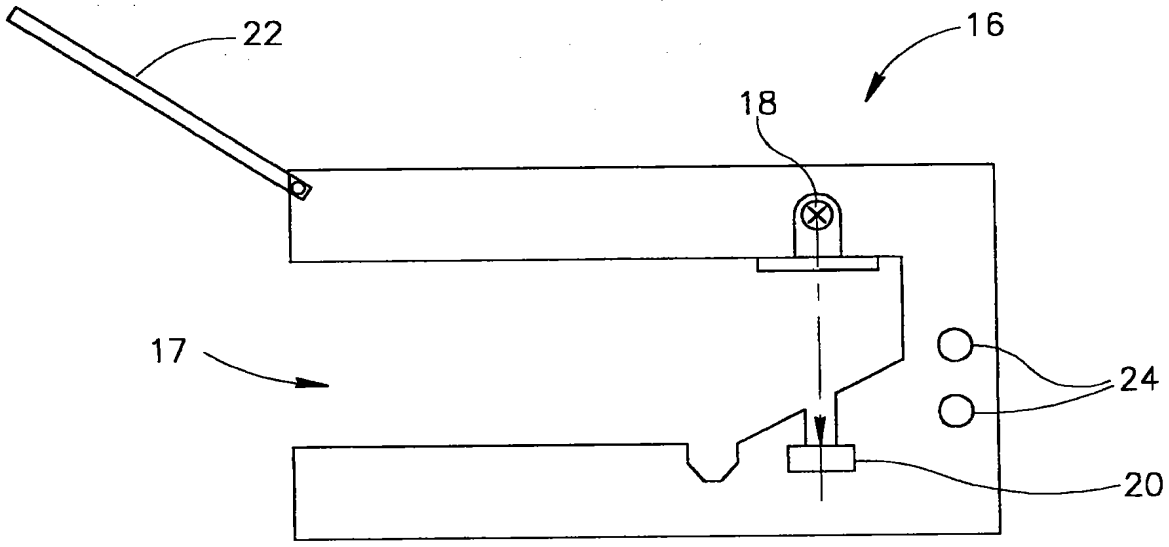

FIG.2

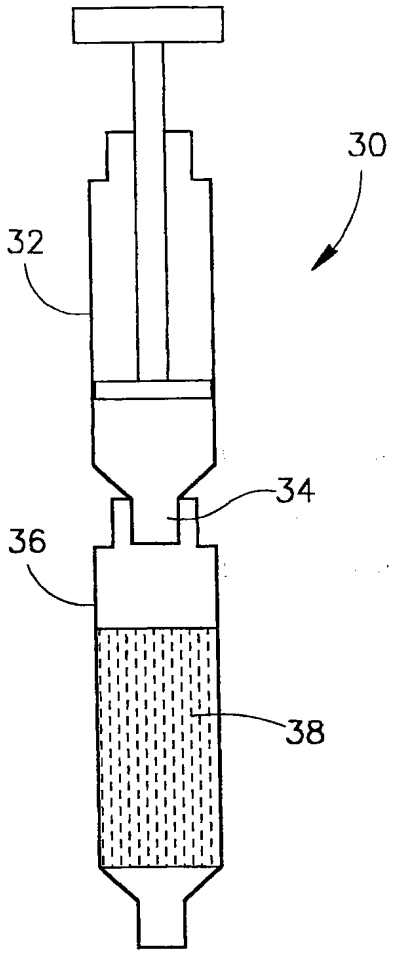

FIG.3

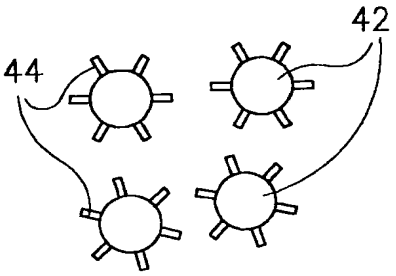

FIG.4A

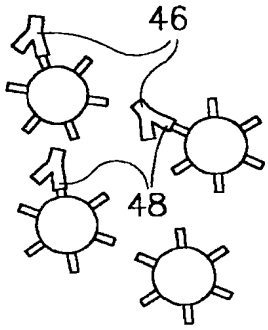

FIG.4B

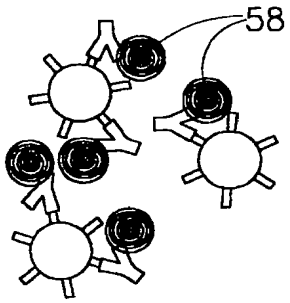

FIG.4C

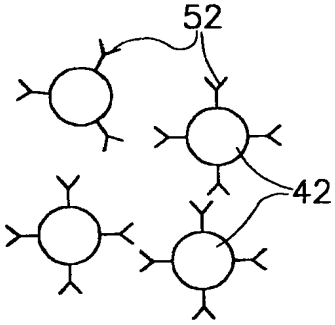

FIG.5A

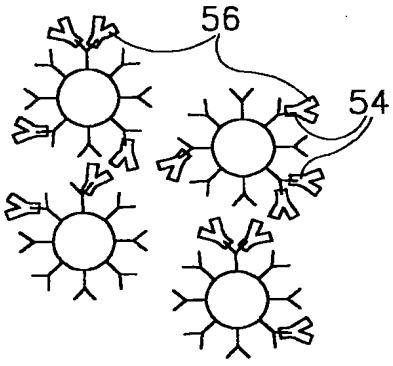

FIG.5B

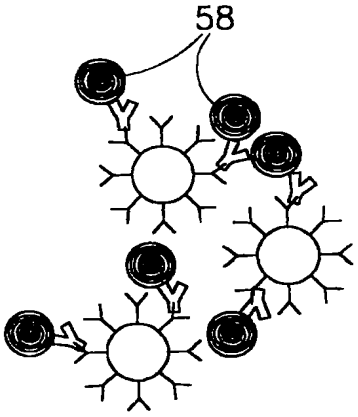

FIG.5C

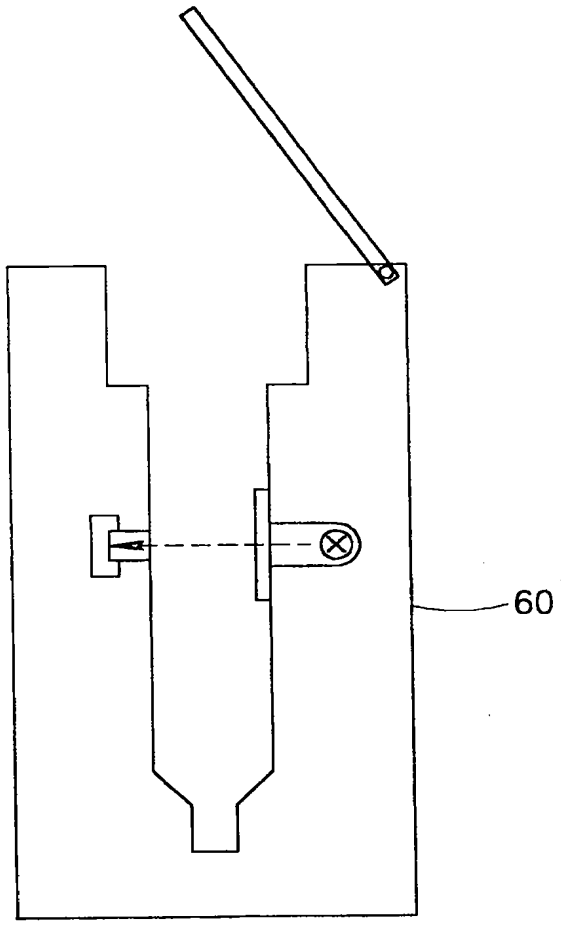

FIG.6

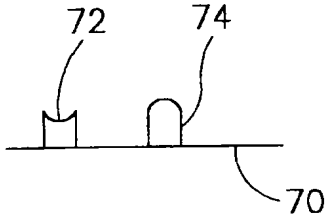

FIG.7A

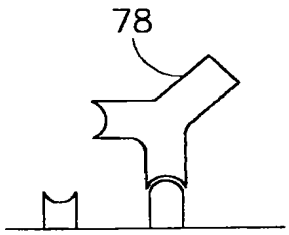

FIG.7B

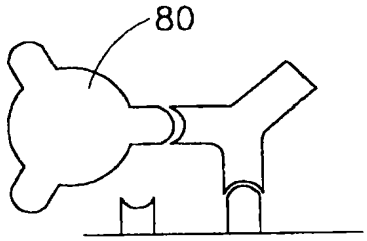

FIG.7C

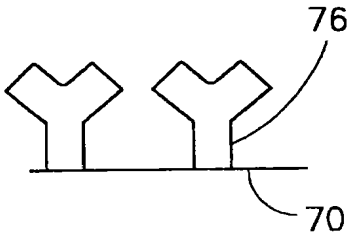

FIG.8A

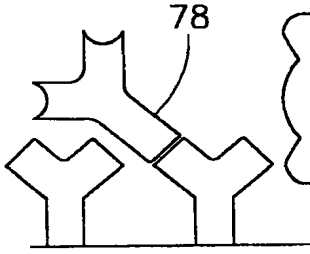

FIG.8B

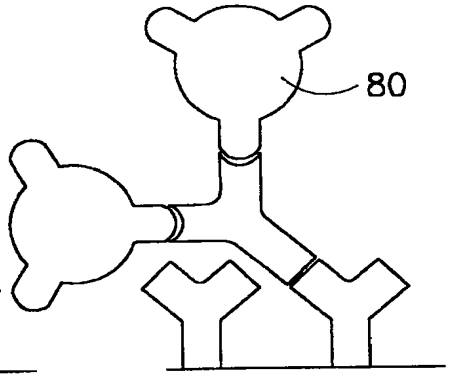

FIG.8C

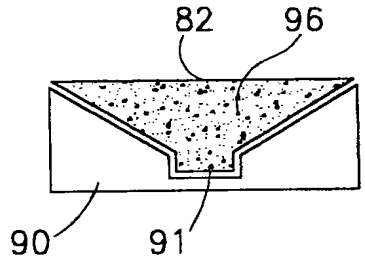

FIG.9A

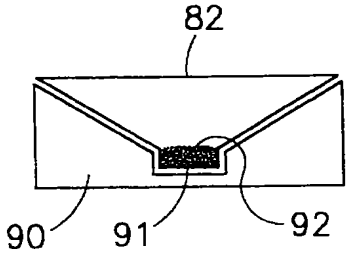

FIG.9B

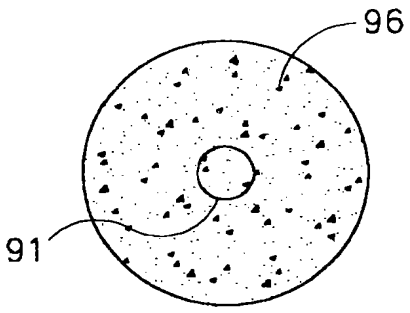

FIG.9C

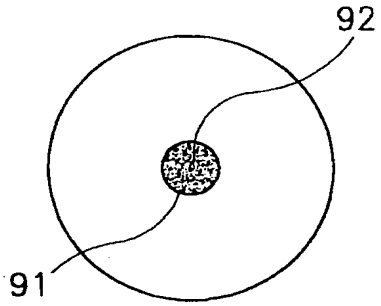

FIG.9D

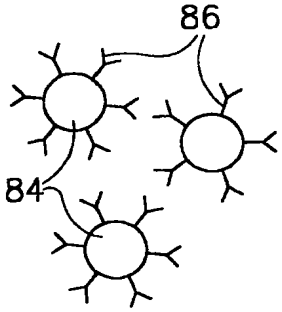

FIG.10A

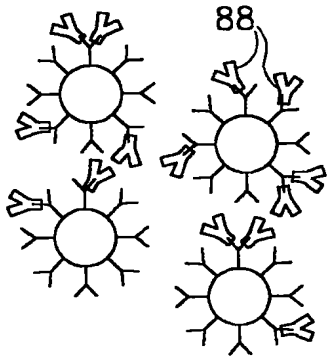

FIG.10B

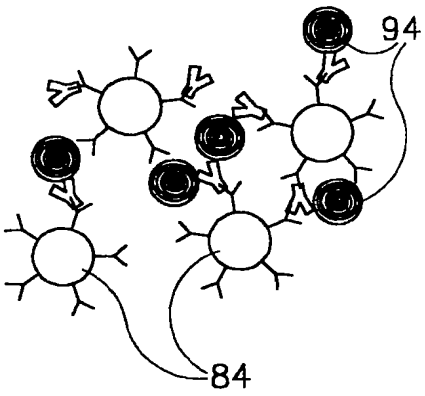

FIG.10C